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File: USPT

Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214554 B1

TITLE: Chronic fatigue syndrome diagnosis

DEPR:

Full-length human RNase L cDNA is described by Zhou et al., Cell 72:753-765 (1993), the entire disclosure of which is incorporated herein by reference, and contained as GenBank accession number L10381, also incorporated herein by reference. The glutathione S-transferase (GST) fusion protein strategy was used to obtain purified recombinant, human 80 kDa RNase L required for production of the RNase L polyclonal antibody. GST-RNase L fusion protein was obtained by expression in E. coli according to the procedure described by Sobol et al., J. Biol. Chem. 270:5963-5978 (1995), the entire disclosure of which is incorporated herein by reference. A polyclonal antibody against recombinant, human 80 kDa RNase L was elicited in New Zealand white rabbits by immunization with the highly purified recombinant, human GST-RNase L fusion protein. Serum was prepared before immunization and retained as a control (pre-immune serum). Initial inoculation was performed on day 1 with 100 .mu.g of GST-RNase L mixed with an equal volume of complete Freund's adjuvant. Boosts with 50 .mu.g of GST-RNase L (50% native and 50% heat denatured protein) mixed with incomplete Freund's adjuvant were given at 14, 21, 49 and 84 days. Blood samples for antibody production were drawn at 120, 150, and 180 days, preceded by additional boosts. Following hydrolysis of GST-RNase L fusion protein with human thrombin, RNase L was covalently coupled to the glutaraldehyde activated cartridge (Whatman) according to the manufacturer's specifications. Sodium borohydride was circulated through the column to reduce the glutaraldehyde that was not coupled to RNase L. The rabbit antiserum containing polyclonal antibody to RNase L was circulated through the glutaraldehyde column for 1 hour at room temperature and eluted according to the manufacturer's specifications. The RNase L polyclonal antibody was characterized by Western blotting using extracts of human 293 cells (ATCC CRL 1573) and an E. coli expressed recombinant GST-RNase L fusion protein, as described by Sobol et al., supra.

DEPR:

Chemical synthesis of the 2-5A azido photoprobe, ApAp(8-azidoA), 5'-monophosphorylation with [.gamma.-.sup.32 P]ATP and polynucleotide kinase to produce [.sup.32 P]pApAp(8-azidoA) and photolabeling of 2-5A binding proteins in PBMC extracts were as described by Charubala et al., Helv. Chim. Acta 72:1354-1361 (1989), the entire disclosure of which is incorporated herein by reference. Photolabeling of the 2-5A binding proteins was thus accomplished by incubation of PBMC extracts (100 .mu.g protein), prepared in the absence of added protease inhibitors, with the 2-5A photoprobe [.sup.32 P]pApAp(8-azidoA) (60 .mu.Ci/nmole, 5 .mu.Ci) (30 min, 4.degree. C.), followed by UV irradiation (8000 watts/cm.sup.2, 30 seconds, 0.degree. C.). The photolabeling mixture was combined with affinity-purified RNase L polyclonal antibody (24 jig protein), Protein A-Sepharose (30 .mu.l) and 100 .mu.l phosphate-buffered saline (PBS), and the mixture was rotated for 1 hr at 4.degree. C. After three PBS washes, the resin was mixed with 40 .mu.l of protein solubilization solution, boiled for 5 minutes and centrifuged (10,600.times.g, 5 min., room temperature). The supernatant was fractionated by 10% SDS-PAGE. The azido-photolabeled immunoprecipitated 2-5A binding proteins were visualized by autoradiography of the dried gel. The combined azido photolabeling/immunoprecipitation methodology eliminates proteins which

immunoreact with the polyclonal antibody to RNase L, but are not 2-5A binding proteins, and also eliminates 2-5A binding proteins which are not immunoreactive to the polyclonal antibody to RNase L.